

**R E M A R K S/ARGUMENTS**

In the Office Action mailed October 15, 2007, 2007, the Examiner rejected claims 1-18 under 35 U.S.C. 112, for different informalities.

Examiner rejected Claim 1 and 16-18 under 35 U.S.C. 102(b) as anticipated by Gutcho (US 2,820,780), and also claims 16-18 under 35 U.S.C. 102(a) as anticipated by Fahey (US 6,780,418). Examiner also rejected claims 1-15 under 35 U.S.C. 103(a) as unpatentable over Gutcho in view of Fahey.

To clarify the subject matter of the invention, Applicants amended paragraph [0014] of the specification by changing R'SSR to R"SSR. This is a correction of an obvious error and is clearly required, because R' is derived from the thiol R'SH and is different from R" of the second mixed disulphide. The R" group of the second mixed disulphide is described in further detail below.

Applicant cancelled claims 3, 4, and 10, and amended claims 1, 5-9, 11-18 to clarify the subject matter of the application.

In making these revisions, care has been taken to ensure that no new matter has been added.

Claims 1-2, 5-9, and 11-18 are now pending in this application.

The Applicant has substantially revised the wording of claim 1 in the light of the rejections raised by the examiner. Claim 1, as now amended, includes the following seven essential features:

- (1) a method of isolating a thiol R'SH from a thiol-containing mixture which includes steps of;
- (2) forming a mixed disulphide R'SSR of the thiol R'SH by reacting the thiol R'SH in the mixture with a second mixed disulphide R"SSR;
- (3) R of the mixed disulfide R"SSR is a hydrophobic moiety which is not immobilised on a stationary phase;

- (4) R" (which was introduced into the specification and original claim 6 because of the error described above) is selected so that the forward reaction of the R'SH with the second mixed disulphide R"SSR to form the desired R'SSR is favoured over the reverse reaction of R'SH with R'SSR back to R"SSR and R'SH;
- (5) purifying the mixed disulphide R'SSR by a process selected from selective precipitation and chromatography;
- (6) reacting the purified mixed disulphide R'SSR with a reducing agent to produce a mixture of the thiols R'SH and RSH;
- (7) separating the mixture of thiols R'SH and RH to isolate the thiol R'SH.

The amendments to claim 1 all find support in the specification and claims as originally filed as discusses further below.

Applicant appreciates the time and consideration provided by Examiner in reviewing this application, however, respectfully traverses the rejection of pending claims at least for the following reasons.

## 2. **Rejection under 35 U.S.C. 112**

- 2.1 The Examiner has rejected the pending claims under 35 USC § 112 as being indefinite. The Examiner has objected to the different descriptions of R as set out in claims 1, 6, 11, 16 and 17. The group or moiety R was described in claim 1 as "a non-immobilised hydrophobic moiety", in claim 6 as "a non-polar thiol", in claim 11 as "a polynuclear aromatic group", in claim 16 as "a hydrophobic moiety" and in claim 17 "as a polynuclear aromatic group". The Examiner has also objected to claims 1-18 because the method of reducing the purified disulphide does not specify any process steps. The Applicant understands that the reference to claims 1 – 18 should be a reference to method claims 1 -15.

- 2.2 In order to clarify the subject matter of the claims, Applicant amended the claims as follow:

In method Claim 1, R is defined as a "hydrophobic moiety which is not immobilized on a stationary phase" and in dependent Claim 11 the "hydrophobic moiety" of Claim 1 is further defined as "a substituted or unsubstituted polynuclear aromatic group". Claim 6 is amended to delete the reference to R.

In amended compound claim 16, R is defined as "a substituted or unsubstituted polynuclear aromatic group" (as in method claim 11). In dependent compound claim 17 the "substituted or unsubstituted polynuclear aromatic group" of claim 16 is further defined as "a substituted naphthyl group" and in dependent compound claim 18 the "substituted naphthyl group" of claim 17 is further defined as a 6-hydroxynaphthyl group.

- 2.3 These changes clearly describe the group R in claims 1, 11, 16 and 17.
- 2.4 The characterisation of the R moiety in amended claim 1 as "a hydrophobic moiety which is not immobilized on a stationary phase" distinguishes the invention from prior art methods which use resins to immobilize the mixed disulphide. This amendment finds support in paragraph [0051] of the specification.
- 2.5 In addition, claim 1 has been further clarified by specifying that the mixed disulphide R'SSR is produced by reacting the thiol R'SH with a second mixed disulphide R"SSR in which the R" moiety is selected so that the forward reaction of the R'SH with the second mixed disulphide R"SSR to form the desired mixed disulfide R'SSR is favoured over the reverse reaction of R"SH with R'SSR back to R"SSR and R'SH. An example of such an R" moiety is given in claim 13 in which R" is defined as a 2-thiopyridyl group. Support for the chemistry underlying this clarifying amendment is found in paragraph [0042]. In essence the group R" is selected so that the corresponding thiol R"SH is non-reactive so that the reverse reaction is not favoured. The thiol 2-pyridinethiol is an example of such a

compound because of stabilisation of the thiolate anion by delocalization into the pyridine ring and the formation of the pyridinethione structure.

- 2.6 Claim 1 has further been clarified by incorporating the wording of claims 3 and 4 and by specifying that the isolating step is a separating step.
- 2.7 The Applicant accordingly submits that the clarifying wording, which has been added to the claims, and, particularly, to amended claim 1 overcome the rejection under 35 U.S.C. §112.

3. **Rejection under 35 U.S.C. 102**

- 3.1 The Examiner has rejected method claim 1 under 35 U.S.C. §102 as being anticipated by Gutcho (US 2,820,780).
- 3.2 Gutcho provides a method whereby disulphides can be reduced to their thiol forms by hydrogen sulphide and metal sulphides. The method can be used to remove disulfides from mixtures containing disulfides. The method of Gutcho is based on the discovery that metal sulphides such as those of bismuth, lead, and mercury and other acid-insoluble metal sulphides, catalyze a reaction between hydrogen sulphide and organic disulphides in an aqueous medium. Gutcho specifically discloses the reduction of the disulphides of cystine, peptides of cystine and proteins containing cystine. The chemistry disclosed in Gutcho is well known and is more than fifty years old.
- 3.3 Referring to the essential features of claim 1 of the present case, as set out in paragraph 1.3 above, Gutcho arguably discloses a method of isolating a thiol R'SH from a thiol containing mixture (feature 1). However, Gutcho does not disclose the prior step of forming a mixed disulphide R'SSR of a thiol R'SH by reacting the thiol R'SH in the mixture with a second mixed disulphide R''SSR in which R is a hydrophobic moiety which is not immobilised on a stationary phase (features 2 – 4). Gutcho also does not disclose purifying the mixed disulphide R'SSR by a process selected from selective precipitation and chromatography

- (feature 5). Gutcho does disclose the reduction of a mixed disulphide with a reducing agent (hydrogen sulphide and a metal sulphide) and, arguably, separating the thiols produced (features 6 and 7). However, essential feature 2 - 5 of claim 1 are not disclosed in Gutcho and Gutcho therefore clearly does not anticipate claim 1.
- 3.4 The Examiner has further rejected compound claims 16-18 under 35 U.S.C. §102(a) as anticipated by Gutcho and under 35 U.S.C. §102(b) as anticipated by Fahey (US 6,780,418).
- 3.5 Amended compound claims 16 – 18 are directed to a disulfide of formula R'SSR in which R' is mycothiyl and R is a non-immobilised substituted or unsubstituted polynuclear aromatic moiety.
- 3.6 Gutcho discloses disulphides of amino acids and peptides such as cystine, diglutathione, diglutamylcystine and cystinyldiglycine. The examples of Gutcho disclose the oxides glutathione (GSSG), cystine and 2,2'-hydroxy-6,6'-dinaphthylidisedisulphide. Gutcho does not disclose a disulphide of the type R'SSR in which R' is a mycothiyl and R is a hydrophobic polynuclear aromatic hydrocarbon moiety as claimed in claims 16 – 18.
- 3.7 Fahey discloses methods and reagents for the detection of mycothiol and mycothiol precursors. In particular Fahey describes a methodology for the attachment of mycothiol via its reactive thiol group to larger molecules, which can be immobilized to allow immunochemical detection of the unique glucosaminylinositol pseudodisaccharide structure of mycothiol. The means by which mycothiol is either biotinylated or attached to ovalbumin is such that it cannot readily be reversed (as is done when relying on thiol-disulfide equilibria), and the purpose of Fahey is not to isolate mycothiol, but to immobilize it in order to detect it by relying on the amplification that forms the basis of many immunochemical procedures.
- 3.8 Fahey does not disclose a disulphide R'SSR in which R' is mycothiyl and R is a non-immobilised hydrophobic substituted polynuclear aromatic hydrocarbon moiety.

- 3.9 Applicant respectfully submits that neither Fahey nor Gutcho discloses or suggests a disulphide of mycothiol and a non-immobilised hydrophobic substituted polynuclear aromatic hydrocarbon moiety. Accordingly, claims 16-18 are novel in view of the cited prior art.

**4. Rejection under 35 U.S.C. 103(a)**

- 4.1 The Examiner has rejected method claims 1-15 as being unpatentable over 35 USC § 103 (a) Fahey in view of Gutcho.
- 4.2 As described in paragraph 3 above, at least the essential features 2 – 5 of claim 1 are not disclosed in Gutcho. Referring again to the essential features of claim 1 as set out in paragraph 1.3, it could be argued that Fahey discloses a method of isolating mycothiol from a mixture containing mycothiol by immobilizing the micothiol in order to detect it (feature 1). However, Fahey does not disclose the prior step of forming a mixed disulphide R'SSR of the micothiol by reacting the mycothiol in the mixture with a second mixed disulphide R"SSR in which R is a hydrophobic moiety which is not immobilised on a stationary phase (features 2 – 3). Fahey does disclose the use of 2-thiopyridine as an activating group for binding the mycothiol to a substrate, but this chemistry for immobilizing mycothiol is well known to the Applicant and predates Fahey. Fahey also does not disclose purifying the mixed disulphide R'SSR by a process selected from selective precipitation and chromatography, reacting the purified mixed disulphide with a reducing agent to produce a mixture and separating the mixture of thiols, to isolate the thiol R'SH (features 5 – 7).

Accordingly, at least features 2 – 5 of claim 1 are not disclosed by Gutcho and at least features 2 – 3 and 5 – 7 of claim 1 are not disclosed by Fahey.

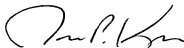
One of the most important features of the present invention is the use of a mixed disulphide, which has a hydrophobic character, early in the process. This protects the thiol from oxidation early in the isolation process and facilitates isolation and purification.

A further important feature of the invention is the use of a non-immobilised moiety to form the mixed disulphide. This allows ready purification, for example, by chromatography. Fahey provides a method of detecting mycothiol by immobilising the mycothiol but does not disclose any way to isolate the compound (which is the primary purpose of the present invention). Gutcho provides a method of reducing disulphides, which method may or may not be applicable in the reduction step of the present invention.

However, neither Gutcho nor Fahey discloses the essential features 2 and 3 of the present invention, namely forming a mixed disulphide of a thiol to be isolated using a second mixed disulphide, in which the mixed disulphide includes a hydrophobic moiety, which is not immobilised on a stationary phase and neither Gutcho nor Fahey suggest features 2 and 3. If anything, Fahey points away from a non-immobilised moiety.

Applicant respectfully submits that pending claims 1, 2, 5-9, and 11-18 as filed and recently amended are novel and patentable in view of the prior art, and the application is now in condition for allowance, which allowance is respectfully solicited.

Respectfully submitted,  
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